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Meloidogyne javanica and *Pratylenchus vulnus* on pecan *(Carya illinoensis)*

Jorge PINOCHET *, Rodrigo RODRIGUEZ-KABANA **, Joan MARULL * and Edward F. McGAWLEY ***

* *Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, IRTA, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, España;*
** *Department of Plant Pathology, Auburn University, Auburn, AL 36849, U.S.A.;*
and *** *Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LO 70803, U.S.A.*

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Summary – Seedlings of eight pecan cultivars grown in the southeastern part of the United States were evaluated for their reaction against *Meloidogyne javanica* and *Pratylenchus vulnus*. Studies were conducted under greenhouse conditions during a three month period following inoculation of 2000 and 1000 nematodes per plant, respectively. Seedlings from the cultivar 4-Po were resistant to *M. javanica* and differed from the rest of the tested materials. Stuart seedlings were moderately resistant, while those from Success, Desirable, Candy and Schley, were susceptible. Candy seedlings had the highest level of parasitism with 940 nematodes per gram of root. Parasitism by *M. javanica* in susceptible trees did not induce visible gall formation, although typical giant cells that spread into the vascular elements were formed. Occasionally, incipient galling was detected. Seedlings from all eight pecan cultivars tested against *P. vulnus* were hosts to the nematode, although seedlings of Candy, Cheyenne, Schley, and Cape Fear were more suitable. Cape Fear seedlings supported the highest nematode populations at 90 days (35 450 nematodes per plant) as well as the highest level of parasitism (2610 nematodes per gram of root). This nematode colonized extensively the cortical parenchyma producing small cavities and discoloration of invaded tissue.

Résumé – Meloidogyne javanica *et* Pratylenchus vulnus, *parasites du pacanier* (Carya illinoensis) – Les réactions de huit cultivars de pacanier cultivés dans le Sud-Est des Etats-Unis vis-à-vis de *Meloidogyne javanica* et *Pratylenchus vulnus* ont été évaluées. L'étude, en serre, a duré 3 mois après l'inoculation avec 2000 (*M.j.*) et 1000 (*P.v.*) nématodes par plant. Les plants du cv. 4-Po se montrent résistants à *M. javanica*, à l'opposé des autres cvs testés : le cv. Stuart montre une résistance modérée, tandis que les cvs Success, Desirable, Candy et Schley sont sensibles. Le plus fort degré de parasitisme est observé chez le cv. Candy (940 nématodes/g racines). Le parasitisme de *M. javanica* sur les plants sensibles ne conduit pas à la formation de galles visibles, même si des cellules géantes typiques, s'élargissant dans les éléments vasculaires, sont formées; occasionnellement, la formation de galles naissantes a pu être détectée. Les plants de tous les cvs testés vis-à-vis de *P. vulnus* se sont révélés hôtes, les cvs Candy, Cheyenne, Schley et Cape Fear étant les plus facilement infectées. Les plants du cv. Cape Fear contiennent la population du nématode la plus élevée (35 450 nématodes par plant, après 90 jours) et montrent le plus fort degré de parasitisme (2610 nématodes par g/racines). *P. vulnus* colonise extensivement le parenchyme cortical, produisant des petites cavités et un brunissement des tissus envahis.

Key-words : *Carya*, host parasite relationship, host suitability, *Meloidogyne*, *Pratylenchus*.

Pecan, *Carya illinoensis* (Wang) K. Koch., is a member of the walnut family (Juglandaceae). It is the most important nut producer of the hickory genus *Carya* and the principal orchard species native to temperate North America. Its production is the basis of a considerable industry in the southeastern United States. It is also cultivated to a limited extent in Mexico, Australia, South Africa, and Israel, and is of recent interest in Spain. Rootstocks for propagating nursery trees are currently grown from seeds (Hagler & Shumack, 1980). Any good quality seedling nut can be used to which pecan cultivars are budded or grafted. Selected clonal rootstocks are not yet available for pecans.

There is ample information on pecan pests, including fungal and bacterial diseases, insects and weeds (Goff *et al.*, 1989). However, there are few references to specific plant parasitic nematodes causing damage to pecans. Payne and Johnson (1979) described the symptomatology caused by lesion nematodes and speculated on the possible incidence of these nematodes in the light soils prevailing in the southern states, while recognizing the lack of research in this area. More recently, Thompson and Grauke (1990) mentioned that root-knot nematodes may cause problems in nurseries with deep sandy soils and that infested plants produce unthrifty trees that must be destroyed. There is also an indication that seedstocks may have variable levels of resistance, raising the possibility of selecting for the trait (Grauke & Pratt, 1985). The purpose of this study was to determine the host suitability of seedlings from eight pecan cultivars, to *Meloidogyne javanica* (Treub) Chitwood and *Pratylenchus vulnus* Allen & Jensen, and to describe the host

parasite relationship of these two common nematode pests that also attack stone fruit crops in the southern states (Bertrand, 1989). *P. vulnus* is considered an important pest of walnuts (*Juglans regia* L.) in California (Lownsbery & Serr, 1963; McKelroy, 1972).

Materials and methods

Seeds of eight pecan cultivars from the southeastern part of the United States were provided by the Fruit Tree Program of Auburn University, Auburn, Alabama, and the Department of Plant Pathology of Louisiana State University, Baton Rouge, Louisiana. Seeds of all cultivars were harvested 20 to 25 days before seeding and brought to Spain for testing. They were placed in vermiculite trays and moved to a greenhouse (minimum temperature of 20 °C) to induce germination. Rooted seedlings were then planted in 1.6 l pots containing a 4:1 (v:v) sand (74 % and, 21 % silt and 5 % clay) and peat mixture (pH 7.2) previously pasteurized at 80 °C. The soil mixture had a cation exchange capacity (C.E.C.) of less than 10 meq/100 g of soil. Plants were kept in the greenhouse for 60 days before inoculation.

Both nematode species were originally from Cabrils, Barcelona, Spain. The population of *M. javanica* was isolated from fig, (*Ficus carica* L.) and increased on tomato (*Lycopersicon esculentum* Mill.), cv. Roma from single egg mass cultures. The *P. vulnus* population was isolated from rose (*Rosa multiflora* L.) and reared monoxenically on carrot cultures (Moody *et al.*, 1973).

Two experiments were conducted. In the first, seedlings approximately 20 cm high (three to five leaves) of the pecan cvs Desirable, Success, Candy, Schley, Stuart, and 4-Po (also known as Perfect Purple Papershed Pecan) were inoculated with a suspension of 2000 eggs of *M. javanica* per plant. Inoculum was prepared by macerating infested tomato roots in a 0.12-0.15 % solution of NaOCl (Hussey & Barker, 1973).

In the second experiment, seedlings of the pecan cvs Desirable, Cape Fear, Candy, 4-Po, Schley, Stuart, Success and Cheyenne, were evaluated using a population of *P. vulnus*. Inoculation level was 1000 nematodes per pot. For inoculum preparation, nematodes in monoxenic culture jars were recovered in distilled water and diluted for aliquot preparation. Inoculation was the same way as for *M. javanica*.

Plants were harvested 90 days after inoculation. The numbers of nematodes per plant (soil and roots), the numbers of nematodes per gram of root, and number of egg masses per root system were determined for *M. javanica*. Nematodes in soil were recovered by the method described by Marull and Pinochet (1991) and then extracted by differential sieving and sugar flotation (Jenkins, 1964). Nematodes in the roots were extracted in the same manner as inoculum, although in this case, root systems were weighed, cut to small pieces and macerated in a blender (Marull & Pinochet, 1991). The resistance rating of each cultivar was estimated based on nematode reproduction and the number of egg masses per root system : R = resistant (no reproduction of the nematode in the plant); MR = moderately resistant (low reproduction of nematode in the plant, Pf/Pi less than 1.5 and less than five eggs masses per root system); S = susceptible (nematode reproduces well in a short period with abundant egg masses in the roots).

The *P. vulnus* population in the soil was extracted in the same way as *Meloidogyne*. Nematodes in the roots were extracted by macerating root tissue without the NaOCl. A host, non host rating was established : NH : non host = Pf/Pi < inoculation level ; PH : poor host = Pf/Pi > slightly higher than inoculation level (up to 1500 nematodes per plant); H : host = nematode reproduces well (more than 1500 nematodes per plant).

Plants were watered daily and fertilized with a full strength Hoagland's nutrient solution once a week (Hoagland & Arnon, 1950). Experiments were conducted under greenhouse conditions with controlled temperature that fluctuated between 20 to 31 °C. Inoculated pots were placed in a sand bed to avoid temperature and humidity fluctuations. In the experiment inoculated with *M. javanica*, each material was replicated seven times in a completely randomized design, while in the experiment with *P. vulnus* each cultivar was replicated 5 times. In both experiments data were analyzed by a One Way Analysis of Variance. Final nematode population, nematodes per gram of root, and number of egg masses per root system data were log_{10} transformed $(x + 1)$. Means were compared by Duncan's Multiple Test ($P < 0.05$).

To complement the host suitability study, histological preparations were made to observe the effects of nematodes in root tissues. Selected root pieces of the cvs Candy, Cape Fear, Cheyenne, Schley and Desirable were washed free of soil particles, fixed in FAA, dehydrated in a tertiarybutyl alcohol series, embedded in a 58 °C melting point paraffin wax and sectioned in a microtome at 15-18 μm. Sections were stained with safranin and Fast-Green.

Results

The cultivar 4-Po was resistant to *M. javanica* and differed significantly from the rest of the tested materials. It showed the lowest final nematode population, nematodes per gram of root and an absence of egg masses in the root systems at the end of the experiment (Table 1). Stuart was moderately resistant. Its final population of 1090 nematodes per plant differed from the rest of the susceptible cultivars. It also differed in the number of nematodes per gram of root and number of egg masses per root system from the resistant 4-Po and the susceptibles Candy and Schley. The cvs Success, Desirable, Candy and Schley showed varying degrees of susceptibility with no significant differences among

Table 1. Total population, nematodes per gram of root, and number of egg masses per root system of *Meloidogyne javanica* in six pecan cultivars at 3 months after inoculation with 2000 nematodes per plant.

Cultivar	Total population (soil and roots)	Nematodes per gram of root	Egg masses per root system	Resistance rating*
4-Po	15 *a*	2 *a*	0 *a*	R
Stuart	1 090 *b*	90 *b*	5 *b*	MR
Success	4 430 *c*	350 *bc*	14 *bc*	S
Desirable	5 690 *c*	370 *bc*	13 *bc*	S
Candy	8 190 *c*	940 *c*	22 *c*	S
Schley	10 050 *c*	690 *c*	23 *c*	S

Data are means of seven replications. Arithmetic means are presented, but data were transformed to $log_{10} (X + 1)$ for analysis. Means in columns followed by the same letter do not differ according to Duncan's Multiple Range Test ($P < 0.05$).

* R = Resistant; MR = Moderately resistant; S = Susceptible.

them. Candy presented the highest level of parasitism with 940 nematodes per gram of root.

The eight pecan cultivars tested against *P. vulnus* were hosts to the nematode, although some cvs, such as Candy, Cheyenne, Schley, and Cape Fear were more suitable. These four differed significantly from 4-Po, and Cape Fear differed from 4-Po and Stuart in the final nematode population per plant (Table 2). There were no differences among cultivars in the number of nematodes per gram of root with exception of Cape Fear which differed from 4-Po, Stuart and Desirable. Cape Fear reached the highest nematode population at 90 days (35 450 nematodes per plant) and the highest level of parasitism (2610 nematodes per gram of root)

The *M. javanica* population used in this evaluation forms undetectable or incipient galls in pecan roots (Fig. 1 A). However, five to eight giant cells of elongated shape were formed generally within the vascular tissues and into the xylem elements (Fig. 1 B). Cells of the endodermis, pericycle and xylem vessels adjacent to giant cells were disorganized and distorted with clear fragmentation of cells walls. Cytoplasma of giant cells appeared dense and granulated with many spherical nuclei. Giant cells normally presented an agglomeration of nuclei. Hyperplasia of cells of the cortical parenchyma and of the endomermis surrounded the nematode for a large area. These cells appeared small, compact, some densely granulated near the cell walls while others contained different size vacuoles that absorbed safranin suggesting that they were dead. In some cases, females established near root meristems. Eggs were laid in a relatively large gelatinous matrix. In all pecan seedlings evaluated, egg masses were completely exposed, easily visible and appeared larger than those formed in other fruit and nut tree hosts, although no measurements were made on their size or content. Histological sections of females with their egg mass seem to confirm this observation (Fig. 1 C).

All seedlings infected with *P. vulnus* with the exception of 4-Po showed clear lesions in young actively growing roots. The nematode colonized extensively the tissues of the cortical parenchyma. Roots contained all stages of the nematode. Cavity formation was present, although reduced comprising only a few cells, probably as a result of early stages of nematode infection. Migration of *P. vulnus* in the cortex was unoriented; thus it was difficult to obtain microtome preparations (16 to 20 μm) showing entire adult specimens (parallel to the stele) in spite of massive colonization. Ruptured cells adjacent to nematode pathways presented dense cytoplasmatic granulation, a defined nucleus stained in grey brownish tones sometimes with visible nucleolus, and a heavy discoloration of the cells walls (Fig. 1 D). The nematode was not detected in meristematic or vascular tissues. No histopathological differences were found between cultivars. The host-parasite relationship of *P. vulnus* in pecan appears to be similar to that of other woody hosts (Corbett, 1974).

Discussion

Parasitism by *M. javanica* in susceptible cultivars did not induce visible gall formation. Occasionnally, incipient galls could be detected. Thus, the galling index normally used to determine the level of susceptibility in root-knot nematode hosts is apparently a poor indicator

Table 2. Total population and nematodes per gram of roots of *Pratylenchus vulnus* in eight pecan cultivars at three months after inoculation with 1000 nematodes per plant.

Cultivar	Total population (soil and roots)	Nematodes per gram of root	Host rating
4-Po	3 000 *a*	430 *a*	H*
Stuart	5 610 *ab*	570 *a*	H
Success	8 140 *abc*	1 130 *ab*	H
Desirable	10 970 *abcd*	540 *a*	H
Candy	19 540 *bcd*	1 660 *ab*	H
Cheyenne	21 510 *bcd*	1 410 *ab*	H
Schley	21 630 *bcd*	1 240 *ab*	H
Cape Fear	35 450 *cd*	2 610 *b*	H

Data are means of five replications. Arithmetic means are presented, but data were transformed to $log_{10} (X + 1)$ for analysis. Means in columns followed by the same letter do not differ according to Duncan's Multiple Range Test ($P < 0.05$).

* H = host.

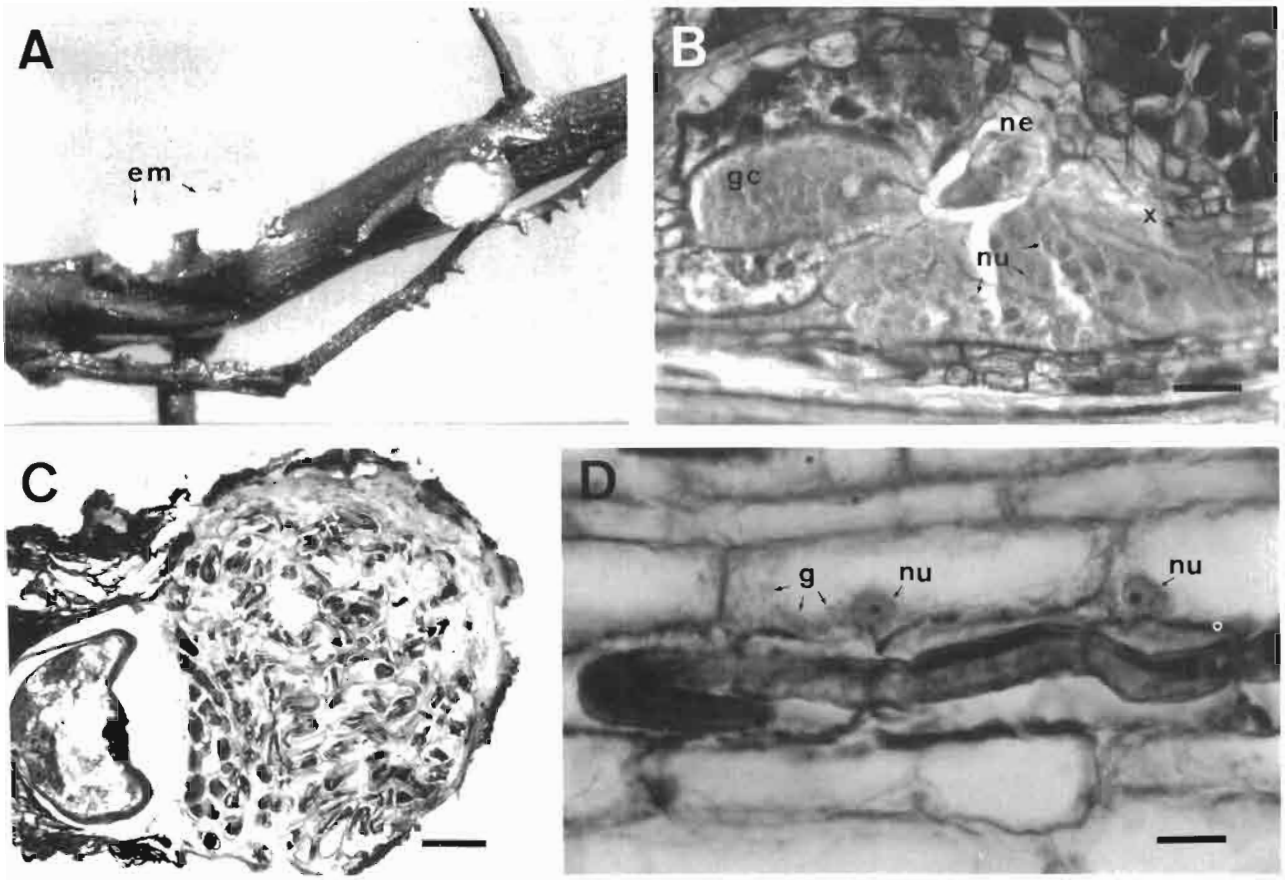

Fig. 1. Parasitism by *Meloidogyne javanica* and *Pratylenchus vulnus* on pecans. A : Pecan root of a seedling cv. Candy showing egg masses of *M. javanica* and an absence of root galling; B : Head of a mature female of *M. javanica* within the vascular tissues feeding on five to six giant cells in cv. Schley; C : Section of a mature female and egg mass of *M. javanica* with differents stages of egg development on cv. Desirable; D : Female of *P. vulnus* migrating intercellularly in the cortical tissues showing cytoplasmatic granulation and defined nuclei in adjacent cells. (em : egg masses; ne : nematode, gc : giant cells; nu : nucleus, x : xylem elements; g : granulation of the cytoplasma. *Bar scale = 60 µm in B; 100 µm in C; 30 µm in D).*

for pecans. In contrast, white (or stained) egg masses visible under the dissecting scope or with lower magnification should be used together with the final nematode population and the number of nematodes per gram of roots to measure pecan susceptibility. The lack of typical galling by this or other *Meloidogyne* species suggests that damage caused by *Meloidogyne* spp. might have been previously overlooked. This *M. javanica* population was previously used in *Prunus* screening tests (Marull & Pinochet, 1991) in which galls were readily evident.

Cape Fear should be considered the best host of *P. vulnus*. It presented extensive lesions in the root system. The cv. 4-Po supported the lowest final nematode population (3000 nematodes per plant). However, its root system was less vigorous than those of the others and thus, probably supported a lower nematode population. The number of nematodes per gram of root (430) at 90 days were high enough to consider 4-Po as a good host for the nematode.

The seedlings from 4-Po were resistant to *M. javanica* and were the least parasitized by *P. vulnus*. From the stand point of nematode resistance, it is the most interesting rootstock tested in this investigation. In a prior study conducted in Louisiana by Grauke and Pratt (1985), the cv. Sioux showed promise as a resistant stock to *Meloidogyne* spp. based on the absence of egg masses and galling. In that study, another interesting material (cv. Moore) presented egg masses and questionable galling. In contrast, Johnson *et al.* (1975) in Texas found heavy infestation in Sioux by a population of *Meloidogyne incognita* (Kofoid & White) Chitwood. These three studies suggest a wide range of responses to root-knot species, races or local populations in pecans,

as well as the ability of at least some root-knot species to induce visible galling in some cultivars.

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